TECHNICAL NOTE

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The Presumptive Reagent Fluorescein for Detection of Dilute Bloodstains and Subsequent STR Typing of Recovered DNA*

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ABSTRACT: A presumptive reagent for dilute blood detection other than luminol is fluorescein. The sensitivity of fluorescein approaches the sensitivity of detection levels of luminol. The fluorescein detection method offers the advantages of working in a lighted environment, and the reaction persists longer than luminol. A series of diluted bloodstains, ranging from neat to 1:1,000,000, was placed on a variety of substrates. Three sets were made per substrate. One set was exposed to fluorescin, one set was exposed to luminol, and one set served as an uncontaminated control. The fluorescein signal persisted longer than luminol. However, background staining for fluorescein was observed on some substrates within 30 s to 1 min, and no background staining was observed for luminol. Stains on non-absorbent surfaces were detectable at 1:100,000 dilutions, and stains on absorbent surfaces were detectable usually at no more than 1:100. The sensitivity of detection of fluorescein was comparable to that of luminol in this study. In all cases, where sufficient DNA was recovered, typeable results at all 13 core CODIS STR loci were obtained from treated bloodstains and controls. The results from STR typing indicate that there was no evidence of DNA degradation.

KEYWORDS: forensic science, fluorescin, fluorescein, luminol, blood identification, DNA typing, short tandem repeats

Dilute blood that can not be seen by eye, or blood on surfaces that have insufficient contrast with the blood, may be detected at crime scenes using a presumptive reagent. Luminol is the most commonly used presumptive reagent for identification of diluted (or washed) blood at a crime scene (1–7). Furthermore, the use of luminol does not have a detrimental effect on subsequent DNA analysis (2,4,5,7). Although very sensitive, luminol has certain operational limitations, which are: (1) the luminol reaction must be observed in as dark as possible an environment, making manipulations difficult, and identification relies on one's eyes becoming accustomed to the dark; and (2) the reaction is short-lived, usually lasting only a few seconds. Thus, photography, which may require long film exposures, necessitates additional treatments with luminol to maintain a positive reaction. Additional treatments may obliterate bloodstain patterns and may dilute the sample.

Cheeseman and DiMeo (8) recommend the presumptive reagent fluorescein (9,10) for dilute blood and non-contrasted detection. Fluorescin is the reduced form of fluorescein, and thus, fluorescin is the prepared reagent for analysis. The sensitivity of fluorescein approaches the sensitivity of detection levels of luminol. The fluorescein detection method offers the advantages of working in a lighted environment, and the reaction persists longer than that of luminol. However, no data exist on whether or not fluorescin/fluorescein has any detrimental effects on DNA, particularly analysis of Short Tandem Repeat (STR) loci. Before employing a reagent for blood detection, potential destructive effects on DNA should be assessed. Therefore, a study was carried out to compare fluorescein and luminol detection on a series of diluted bloodstains placed on a variety of substrates and to evaluate the ability to type STRs derived from samples treated with the presumptive test reagents.

Materials and Methods

Blood from a single donor, drawn by venipuncture into an EDTA tube, was diluted 1:10, 1:50, 1:100, and 1:1000 with physiological buffered saline, and in some cases blood was diluted 1:10,000, 1:100,000, and 1:1,000,000. One hundred μL aliquots of neat and diluted blood (one set) were deposited onto various substrates and allowed to dry. The substrates were: carpet, stained wood, unstained wood, black formica, white formica, cinder block, red brick, denim, and leather. Three sets were made per substrate. The samples were maintained at ambient temperature for seven days. One set was used for exposure to fluorescein, one set was used for luminol, and one set served as an untreated control.

Luminol was purchased from Morris-Kopec Forensics, Inc. (Altamonte Springs, FL) and prepared according to manufacturer's recommendation. Fluorescin, the reduced state, was prepared fresh according to Cheeseman and DiMeo (8). Using an aerosol sprayer (Croion Industrial Products, Hebron, IL), each test reagent was applied to the stains such that the entire surface of the stain was covered. Fluorescein (i.e., oxidized form) stained materials were ob-

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served using an alternate light source (450 nm) and orange safety goggles. Luminol stained materials were viewed in the dark with the naked eye.

Blood samples were recovered either by cutting or by swabbing with swabs wetted with distilled water. DNA was extracted according to Comey, et al. (11). The quantity of recovered DNA was determined using the slot blot hybridization assay described by Waye, et al. (12) and Budowle, et al. (11). Amplification of the CODIS core 13 STR loci was carried out using the AmpFℓSTR Profiler Plus™ PCR Amplification Kit and the AmpFℓSTR COfiler™ PCR Amplification Kit (Perkin Elmer Biosystems, Foster City, CA) according to manufacturer's recommendation. Typing was performed by capillary electrophoresis on the ABI Prism™ 310 Genetic Analyzer (Perkin Elmer Biosystems, Foster City, CA) according to the manufacturer's recommendations and Budowle and Moretti (13).

Results and Discussion

In order to investigate the potential effects of the presumptive reagent fluorescein (as well as luminol) on STR analysis, bloodstains on absorbent (carpet, leather, denim), non-absorbent (stained wood, unstained wood, black formica, and white formica), and porous (brick and cinder block) materials were exposed directly to fluorescin or luminol. The positive attributes of using luminol are: (1) the preparation is commercially-available and requires a single application for initial viewing; (2) the preparation of the luminol reagent requires only the addition of water and thus can be prepared in the field; (3) no background staining was observed in our study, and 4) stains on non-absorbent surfaces were detectable at the 1:100,000 dilution, while stains on absorbent surfaces were detectable usually at no more than a 1:100 dilution. Sensitivity levels were similar to those reported by Fregeau, et al. (7). Less desirable attributes of the luminol method are: 1) manipulation of spraying in specified areas is difficult in the dark; and 2) depending on the substrate, the luminol reaction lasted a few to 30 s.

In contrast to luminol: (1) the fluorescin field-ready reagent is not commercially available and requires some laboratory preparation not routinely performed by crime scene investigators. Because of the need for fresh reagent, the use of fluorescin may not be applicable by all investigators as a field deployable tool; (2) visualization of fluorescein-treated bloodstains requires use of an alternate light source set at approximately 450 nm and orange safety goggles; (3) manipulation of spraying was easier because of working under lighted conditions; and (4) the fluorescein reaction persisted longer than luminol. However, background staining was observed on some substrates (i.e., carpet, wood, cinder block, and denim) within 30 s to 2 min. Since a crime scene investigator will never know *a priori* which materials may fluoresce in the presence of fluorescein, identification of potential bloodstains should still be performed in an expeditious manner. Additionally, it is prudent practice to initially test an unstained portion of a substrate, when possible, for potential background staining prior to applying the presumptive reagent for bloodstain detection. Stains on non-absorbent surfaces also were detectable at 1:100,000 dilutions, and stains on absorbent surfaces were detectable usually at no more than 1:100 dilution. Thus, the sensitivity of detection of fluorescein was comparable to that of luminol. Finally, the use of a thickener (Keltrol RD, which is gum xanthan) in the fluorescin preparation was deemed necessary to reduce running and possible mixing of different stains in close proximity.

The main purpose of the study was to determine if DNA could be recovered from fluorescin/fluorescein-treated samples and if the DNA was typeable for STRs. The entire swab or entire stain cutting was extracted in each case. The quantitation of DNA by slot blot hybridization was performed to demonstrate the presence of recoverable DNA and to determine the quantity of sample for the PCR. The quantity of recoverable DNA was not determined exactly, because some variation in recovery of DNA was observed due to swabbing (some sample remained on the substrate in a number of cases) and efficiency of extraction from cuttings can vary; also there was not a sufficient number of samples at each dilution/substrate/treatment for the amount of extractable DNA to be reliably determined. Moreover, the main purpose of the study was to determine whether or not DNA degrades in the presence of fluorescin/fluorescein. Thus, trends on DNA quantity were evaluated. Generally, the amount of DNA recovered was similar (compared at appropriate dilution) for fluorescin/fluorescein-treated, luminol-treated, and untreated samples. DNA typically was detected by slot blot hybridization up to a 1:100 dilution, except for that recovered from denim (approximately 1:10 dilution), cinder block (no detectable DNA at neat or any dilution), red brick (only neat), and unstained wood (approximately 1:10 dilution). Since no DNA was recovered from the untreated cinder block controls, most likely the negative results are due to blood seeping into pores in the substrate and not being readily recoverable by swabbing.

In all cases, where sufficient DNA was recovered, typeable results at all 13 STR loci were obtained, with no evidence of DNA degradation. Regardless of treatment or no treatment, STR typing was possible on non-absorbent surfaces up to the 1:100 to 1:1000 dilutions (except for unstained wood which was typeable up to 1:50 to 1:100 dilutions), on carpet and leather up to 1:50 to 1:100 dilutions, on denim with neat samples and 1:10 dilutions, and on cinder block with neat samples only. Red brick was the only substrate where the untreated sample was typeable at a greater dilution than the treated samples (1:100 dilution versus neat to 1:10 dilution, respectively). It can be expected that evidentiary material purposely or inadvertently contaminated with fluorescin/fluorescein or luminol can be successfully typed, as long as sufficient quantity and quality DNA is recovered.

Greater dilutions of blood than were typeable in this study may be analyzed successfully in practice. The quantity of DNA in each extracted sample was used to determine the volume of sample for the PCR. However, a maximum of 20 μL could be placed in a PCR following the protocol used in this study. No effort was made to concentrate the extracted sample to increase the amount of template DNA for the PCR. Thus, more dilute samples may be typable by concentrating the sample.

In conclusion, presumptive tests/enhancement reagents are needed to detect some bloodstains. In deciding which enhancement reagent to employ, it is desirable to appreciate the utility and limitations of the assay. The results of the current study demonstrate that direct application of fluorescin (or for that matter luminol) on bloodstains does not interfere with the ability to recover typeable DNA. Either reagent, fluorescein or luminol, may be used as a enhancement reagent for bloodstain detection at a crime scene, although a commercially-available, stable fluorescin assay would be desirable. The choice of reagent should be based on operational preferences of the investigator. Also, because of successful STR typing results from DNA obtained from diluted and contaminated bloodstains, the data in our study provide additional support for the reliability of STR typing.

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